

## CLOSED SYSTEM CULTURE OF *PENAEUS VANNAMEI*

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**ABSTRACT** *Penaeus vannamei* were cultured utilizing three different closed recirculating seawater systems. The first system used biological filtration for water treatment. The second system utilized both physical and chemical filtration, but no biological filtration. The third system used a combination of biological, physical, and chemical filtration. Shrimp growth was monitored for a 12-week period for each system. Shrimp from the biological filtration system had a growth rate of 0.82 g/wk and an overall survival rate of 45.6%. Shrimp from the closed system which used physical and chemical filtration had a growth rate of 0.99 g/wk and a survival rate of 29.2%. In the third system which combined both types of filtration, the shrimp growth rate averaged 0.65 g/wk and the survival rate was 56.9%.

### INTRODUCTION

The intensive culture of marine shrimp has been of interest since shrimp farming first began in the late 1960s. Initial grow-out trials at the Bureau of Commercial Fisheries in Galveston, Texas began in raceway tanks in 1972 and continued into 1980. One commercial venture, Intensive Culture Systems (Summerland Keys, Florida), attempted to grow shrimp in intensive closed systems as early as 1974. One of the largest attempts at closed system marine shrimp culture was by Aquabiotics (King James, Inc., Park Forrest, Illinois) in 1979. Currently, there are at least three commercial ventures working with closed systems for the culture of marine shrimp: the Stillman Ranch and Red Ewald in Texas, and Aquamar in Florida. Two research facilities, the University of Texas Marine Science Institute (Port Aransas, Texas) and the Gulf Coast Research Laboratory (Ocean Springs, Mississippi) are researching closed system culture on a small scale.

### MATERIALS AND METHODS

Three grow-out trials were conducted during 1989. The first two trials were run simultaneously and shrimp growth in the two unreplicated systems was compared. Shrimp system 1 (SS-1) utilized biological filtration, while shrimp system 2 (SS-2a) utilized both physical and chemical filtration with no biological filtration. The third trial, shrimp system 3 (SS-3a), utilized a combination of biological, physical and chemical water treatment. All systems were housed in a passively-heated greenhouse.

**Shrimp System 1.** SS-1 (Fig. 1) consisted of a 1.8 m x 7.3 m x 0.28 m rectangular raceway with an area of 13.28 m<sup>2</sup> and a volume of 5.74 m<sup>3</sup>. Wastewater was collected in

a 5.08 cm diameter PVC slotted pipe running the length of the tank and passed by gravity flow through the end wall into a settling tank. The settling tank, 0.93 m x 1.85 m x 0.61 m, was packed with a plastic media, Norpac (Jaeger Products, Inc., Spring, Texas). A chamber at one end provided room for a submersible sump pump (Little Giant 6C1, MR #506913, Oklahoma City, Oklahoma) which supplied water through a 3.81 cm PVC pipe split into three distribution pipes. Two of the pipes were plumbed into the top of a pair of protein skimmers, 0.15 m in diameter and 1.8 m high, sparged with compressed air. Water exited the bottom of the skimmers and was elevated by gravity to two rotating spray bars. Water from the spray bars irrigated two aerobic trickling filters.

The filters were constructed of a synthetic spawning mat material (Anderson Bait Company, Lonoke, Arkansas) wound in a spiral around corrugated fiberglass panels which rested on top of a biological filter. Effluent from the spiral filters trickled into the top of the submerged trickling biological filter. The biological filter was constructed in a 0.96 m x 1.9 m x 0.2 m fiberglass tank. Plastic egg crate louvers suspended off the tank bottom with 2.54 cm diameter PVC pipe served as a support for clam shell and a media of ground PVC. Water flowed down through the media and out a bottom drain into the raceway. A 5.1 cm diameter PVC standpipe maintained water in the submerged trickling biofilter at a depth of 17.8 cm. The third pipe from the pump was directed to a spray bar running the length of the raceway and suspended above the water.

Additional circulation was provided by a ½ hp Jacuzzi pump (Model 5L, Little Rock, Arkansas). The intake pipe was placed inside a circular basket perforated with 1.27 cm square holes and covered with screen. The basket was used to prevent shrimp from being aspirated by the pump. Water passed through the basket into a submerged 2.54 cm diameter spray pipe running the length of the raceway. A venturi injector (Sophisticated Systems, Palm Harbor, Florida)

(Use of trade names does not imply endorsement.)

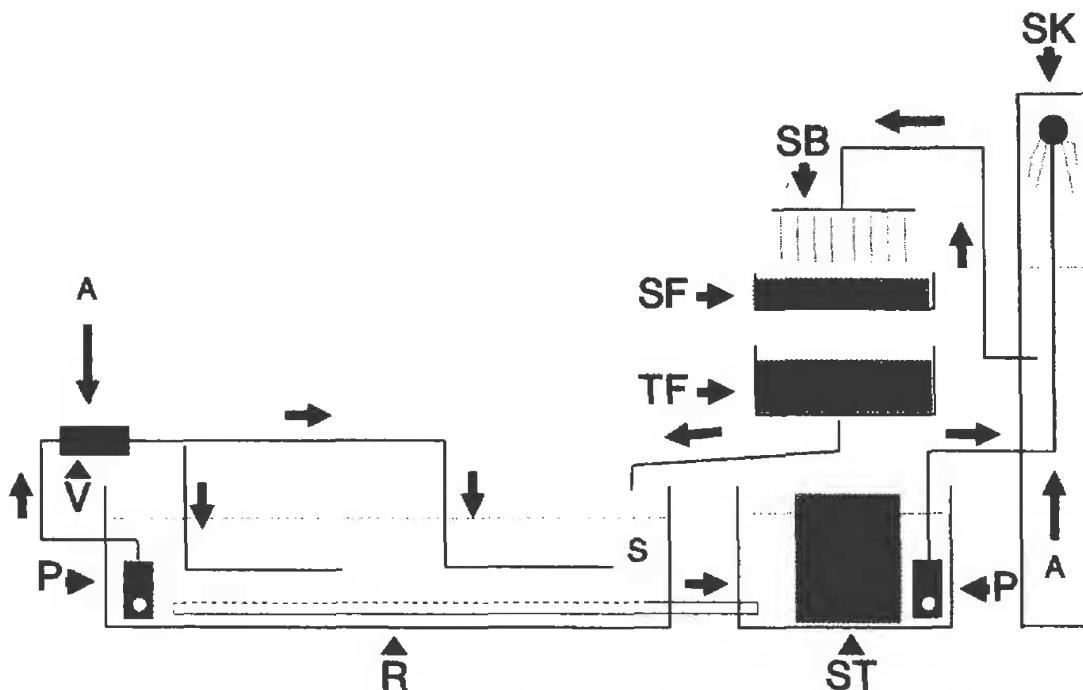


Figure 1. Cross-section diagram of SS-1 utilizing biological filtration: P - pump, V - venturi injector, R - raceway, ST - settling tank, TK - trickling filter, SF - spiral filter, SB - spray bar, SK - skimmer, A - site of air injection, S - site of sample collection.

was installed in the supply pipe to oxygenate the water. Total area of the system including filters was 16.9 m<sup>2</sup> and total volume was 6.6 m<sup>3</sup>. Ten net substrates, 30.5 cm x 152 cm with 0.6 cm mesh openings, were secured across the culture tank to provide shelter. Water flow for SS-1 was 28 gpm with a turnover rate of 23 times per day (Table 1).

In week 11, water lost throughout the study was replaced (80% water change) and the system was cleaned.

**Shrimp System 2a.** A rectangular raceway, 1.8 m x 7.3 m x 0.6 m, a lamellar separator and reservoir box containing six protein skimmers were the main components of SS-2a (Fig. 2). A 7.6 cm slotted PVC pipe running the length of the raceway collected wastewater. The water was pumped (Little Giant, 6CI MR #506913, Oklahoma City, Oklahoma) into the bottom of a lamellar separator inside a 0.76 m x 2.1 m x 0.91 m tank elevated 0.2 m above the floor. Water flowed up through the angled lamellar media and cascaded into the reservoir box, 1.0 m x 0.91 m x 1.3 m, which contained the six protein skimmers.

The protein skimmers were constructed of PVC pipe 10.1 cm in diameter and 1.6 m in length. A 2.54 cm hole located 1.1 m from the bottom of the skimmer allowed

water to flow into the skimmer pipe. Water flowed out the bottom of the skimmer through a 5.08 cm diameter PVC pipe connected to a manifold constructed of 30.48 cm PVC pipe. A 5.08 cm diameter PVC pipe in one end of the manifold connected through the sidewall of the reservoir box to the intake of a Jacuzzi ½ hp pump (Model 5L, Little Rock, Arkansas). Water was directed through a venturi aspirator (Sophisticated Systems, Palm Harbor, Florida) into a submerged spray bar running the length of the raceway. Activated air (ozone) generated from a UV unit (Water Management, Inc., Pascagoula, Mississippi) was sparged into the six protein skimmers and injected into the venturi. Ten 1.5 m x 0.30 m nets were hung vertically across the culture tank to provide additional substrate for shrimp. Water flow for SS-2a was 32 gpm with a turnover rate of 20 times per day. The system had a total area of 13.5 m<sup>2</sup> and a total volume of 8.7 m<sup>3</sup>.

**Shrimp System 3a.** SS-3a was constructed later in 1989 by utilizing some components from the other two systems as noted. The system consisted of four circular tanks, 1.8 m in diameter, 66.0 cm in depth with a capacity of 2,273 L each (Fig. 3). A screened standpipe allowed

TABLE 1  
Physical and biological parameters for three closed systems.

System	SS-1	SS-2a	SS-3a
Area			
total m <sup>2</sup>	16.9	13.5	12.2
filter %	21.3	11.9	14.9
ratio f/t*	0.27	0.13	0.17
Volume			
total m <sup>3</sup>	6.6	8.7	10.1
gallons	1752	2303	2767
filter %	13.5	30.5	33.1
ratio f/t*	0.15	0.44	0.49
Water			
flow (gpm)	28	32	24
turnover/day	23	20	13
exchange %/wk	7.1	1.8	3.3
Stocking			
number	3300	3300	4480
tank			
#/m <sup>2</sup>	248	277	430
#/m <sup>3</sup>	575	500	663
system			
#/m <sup>2</sup>	195	244	367
#/m <sup>3</sup>	497	378	444
Harvest 12 wk			
mean size (g)	11.60	13.02	8.09
growth g/wk	0.82	1.00	0.65
survival %	45.6	29.2	56.9
production kg	12.5	17.4	20.7
kg/m <sup>3</sup>	1.9	2.0	2.0
gal/lb	118	73	88

\*ratio of filter to tank

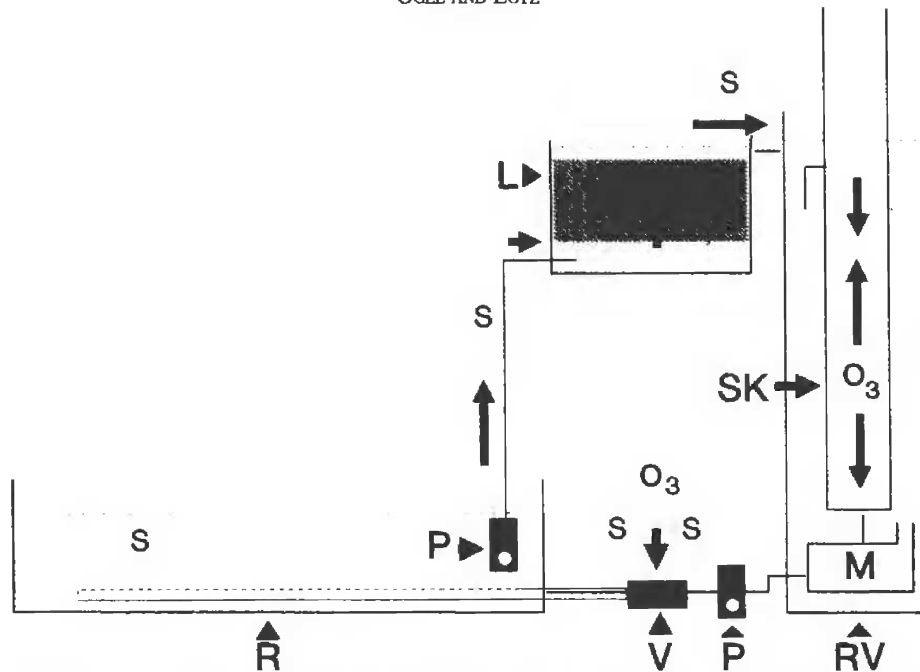


Figure 2. A cross-section diagram of SS-2a utilizing chemical and physical water treatment: R - raceway, P - pump, V - venturi injector, L - lamellar separator, SK - skimmer, RV - reservoir tank, O - site of ozone injection, S - site of sample collection.

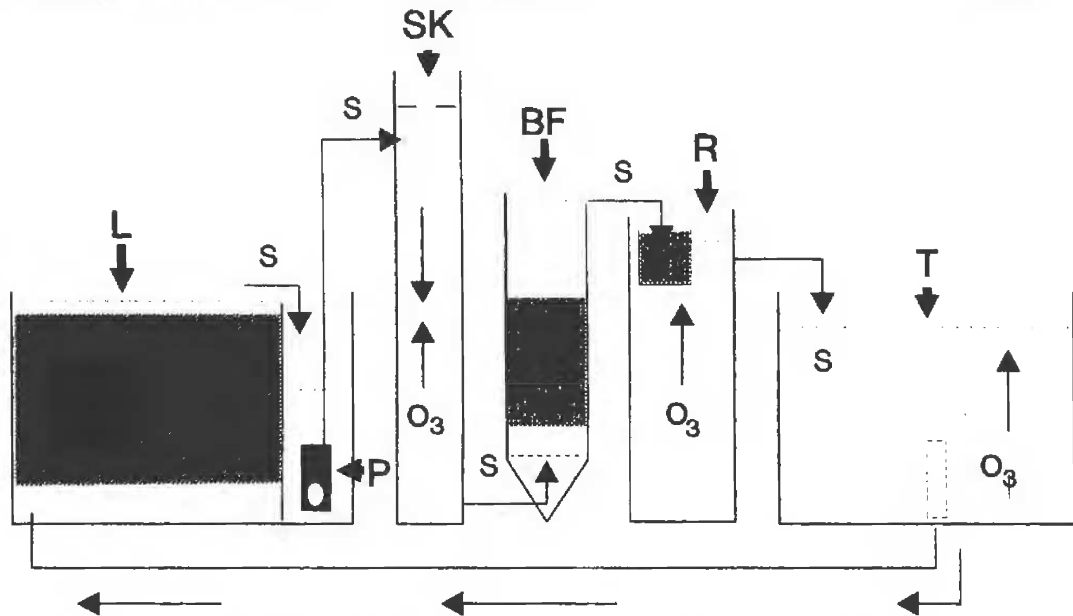


Figure 3. A cross-section diagram of SS-3a utilizing chemical, physical and biological water treatments: L - lamellar separator, P - pump, SK - skimmer, BF - biological filter, R - reservoir tank, T - culture tank, O - site of ozone injection, S - site of sample collection.

water to flow out of the tanks through 5.08 cm center drains where it was directed to the bottom of the lamellar separator used in SS-2a. Water flowed up the inclined media of the separator and overflowed into a pump chamber. The pump chamber, a 81.3 cm x 71.1 cm x 81.3 cm fiberglass tank, had a 3.81 cm PVC bulkhead fitting in the sidewall which connected directly to a ½ hp Jacuzzi pump.

The pump supplied water to the top of a 30.5 cm diameter, 2.4 m tall tank with a conical insert having a 5.08 cm center hole, all of which served as a protein skimmer. Water exited the bottom of the skimmer and entered the bottom of a biofilter. The biofilter was constructed of a conical-bottomed 0.029 m cylindrical tank 1.5 m tall. A diffusor plate with holes on 2.54 cm centers was placed at the top of the conical part of the filter tank. Gravel placed on top of the diffusor retained the carbon used as a filter media. Water flowed up through the filter, which caused some fluidization, where it was collected in an overflow trough and directed to a reservoir chamber used in SS-2a. Water entering the reservoir flowed through a cylindrical plastic perforated basket used in SS-1 containing a bonded filter matting (Fritz Aquaculture, Dallas, Texas). Four separate bulkhead fittings exited the sidewall of the reservoir and directed the water back to the culture tanks.

Flow rates were controlled by valves. Excess water from the reservoir was directed by an overflow pipe back to the pump chamber. Ozone was sparged into the skimmer, the reservoir and directly into each of the four culture tanks. Total area for SS-3a was 12.2 m<sup>2</sup> and total volume was 10.1 m<sup>3</sup>. The flow rate was 24 gpm with a turnover rate of 13 times per day.

Hatchery-reared *Penaeus vannamei* postlarvae, cultured to a minimum size of 1 g, were hand counted into the three systems. SS-1 and SS-2a were stocked in February 1989 with 3,300 shrimp each. SS-3a was stocked in August 1989 with 1,140 shrimp per tank for a total of 4,480 shrimp. Salinity varied from 16 to 20 ppt and temperature ranged from 23 to 28°C.

Shrimp were fed continuously utilizing automatic Zeigler baby belt feeders (Gardners, Pennsylvania). Rangen (Buhl, Idaho) and Ziegler (Gardners, Pennsylvania) artificial shrimp grower feeds were fed to shrimp at a rate of 5% body weight. A sample of no less than 25 animals were individually weighed each week to the nearest 0.01 g on an electronic balance. Feeding rates were adjusted weekly for all systems. Shrimp growth was monitored for a period of 12 weeks, at which time tanks were harvested and survival rates determined. Water quality values for pH and ammonia were determined weekly by using an Orion pH/ion analyzer. Nitrite and nitrate were determined by standard methods (EPA 1983) each week. Sodium carbonate was added as needed in an attempt to regulate pH. Water was added only to replace loss and after occasional flushing of the lamellar separator. In addition, total heterotrophic

bacteria were determined by plating on marine agar and counting colony-forming units. Presumptive *Vibrio* colony-forming units cultured on TCBS agar were counted weekly for SS-1 and SS-2a. Additional weekly water quality samples were taken from the inflow and outflow for each filter component of SS-2a and SS-3a for three weeks prior to the 12-week inventory. After the systems were modified, additional samples were taken for three weeks prior to the 20th-week harvest. The difference between values for inflow and outflow for each component was calculated and expressed as percent instantaneous change for ammonia and for nitrate.

After the 12-week period when shrimp were harvested, SS-2a and SS-3a were modified and labelled as SS-2b and SS-3b, respectively. Additional water quality samples were analyzed from the individual filter components.

For SS-2b, six 0.16 m<sup>2</sup>, 1.5 m high skimmers were plumbed directly into the lamellar outflow (Fig. 4). Water flowed down through the skimmers, out the bottom and was directed back up over the side of the reservoir chamber. SS-3b (Fig. 5) was modified by addition of a hydrocyclone (Flo Trend Systems, Inc, Denton, Texas) for particulate removal. The hydrocyclone was plumbed in line between the pump and the skimmer. The 30.48 cm diameter skimmer was replaced with a 45.72 cm diameter cylinder. The biofilter was replaced with a flat bottomed cylinder with a conical top 61 cm in diameter. Water exited the top of the filter through a 5.08 cm diameter PVC pipe which directed the water through the sidewall of the reservoir.

## RESULTS

Results for the three systems were significantly different. Shrimp growth averaged 0.82, 0.99 and 0.65 g/wk and survival rates for the 12-week period were 45.6%, 29.2% and 56.9% for SS-1, SS-2a and SS-3a, respectively. Total production was 12.5, 17.4 and 20.7 kg or 1.9, 2.0, and 2.0 kg/m<sup>3</sup> for SS-1, SS-2a and SS-3a, respectively (Table 1). Water varied in pH from 7.12 to 7.87 for SS-1, 7.22 to 7.93 for SS-2a, and 7.33 to 7.80 for SS-3a (Fig. 6). Total ammonia fluctuated from 0.01 to 0.254 ppm for SS-1, 0.094 to 0.455 ppm for SS-2a, and 0.083 to 1.06 ppm for SS-3a (Fig. 7). Nitrite fluctuated from 0.098 to 2.12 ppm for SS-1, 0.074 to 2.35 ppm for SS-2a, and 0.063 to 1.38 ppm for SS-3a (Fig. 8). Nitrate increased from 0.118 to 55.3 ppm for SS-1, varied between 1.35 to 23.6 ppm for SS-2a, and ranged from 4.79 to 12.5 ppm for SS-3a (Fig. 9). Nitrate was actually reduced in SS-2a and SS-3a. A comparison was made of the water quality data for systems 2a, 2b, 3a and 3b for the individual filter components. The pump removed ammonia from all systems ranging from 3.2% to 13.5%. The lamellar separator was also removing ammonia except in SS-3a where ammonia was added. The skimmer for SS-2a and 2b also increased the ammonia

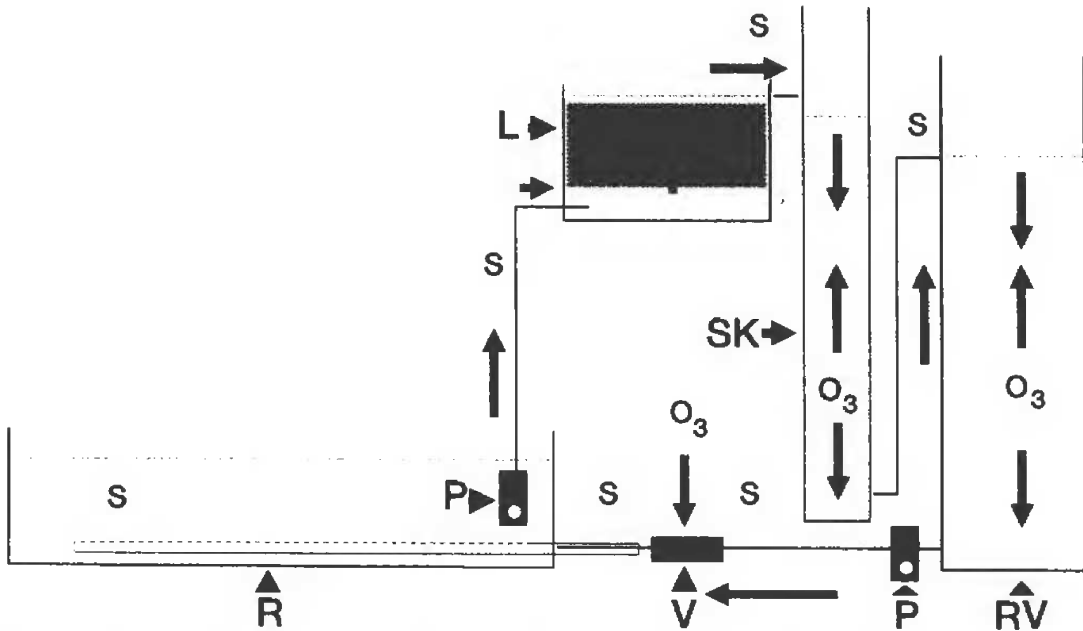


Figure 4. A cross-section diagram of SS-2b after modification: R - raceway, P - pump, L - lamellar separator, SK - skimmer, RV - reservoir tank, V - venturi injector, O - site of ozone injection, S - site of sample collection.

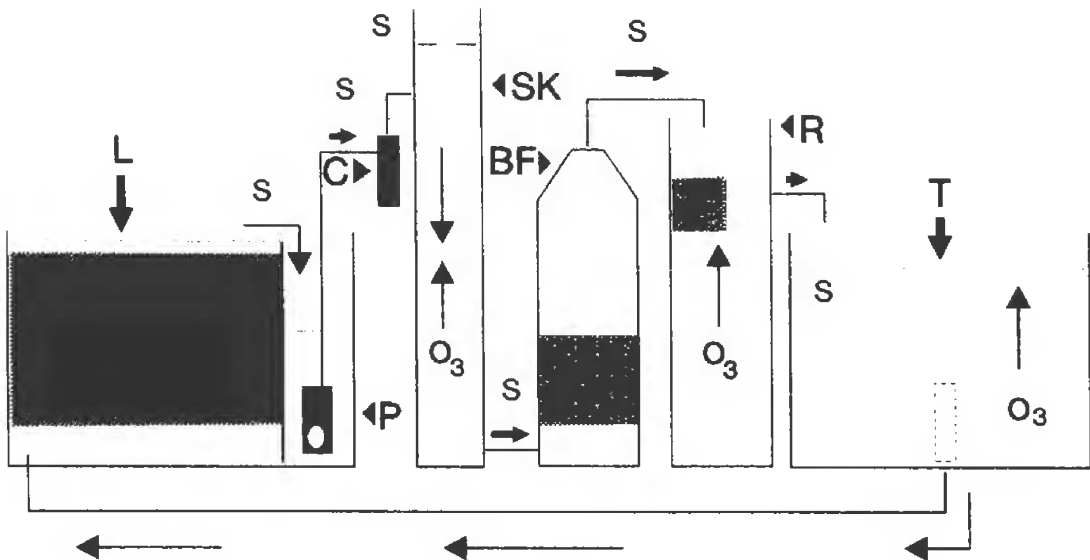


Figure 5. A cross-section diagram of SS-3b after modification: L - lamellar separator, P - pump, C - hydrocyclone particle separator, SK - skimmer, BF - biological filter, R - reservoir tank, T - culture tank, O - site of ozone injection, S - site of sample collection.

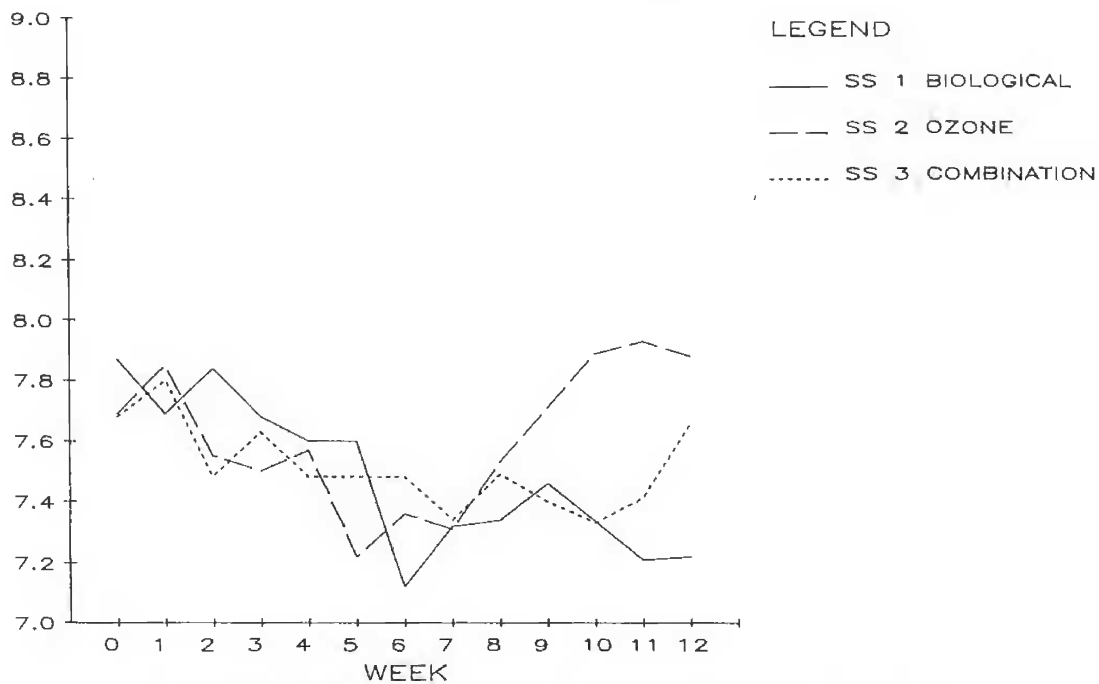


Figure 6. Graph of pH monitored over a 12-week period for three shrimp systems.

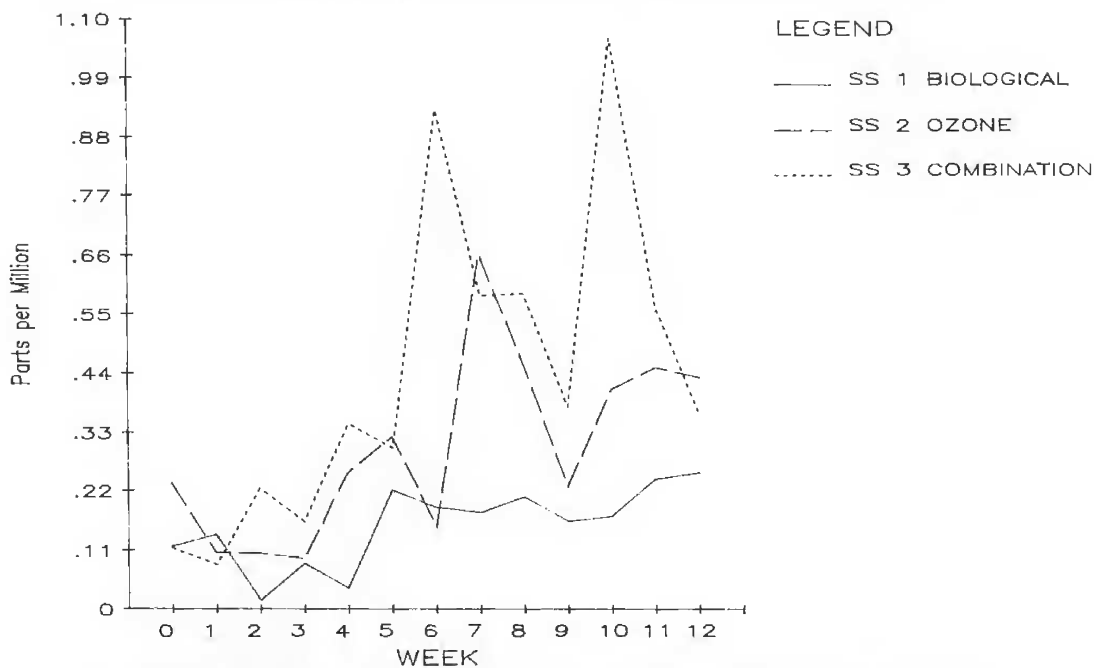


Figure 7. Graph of total ammonia monitored over a 12-week period for three shrimp systems.

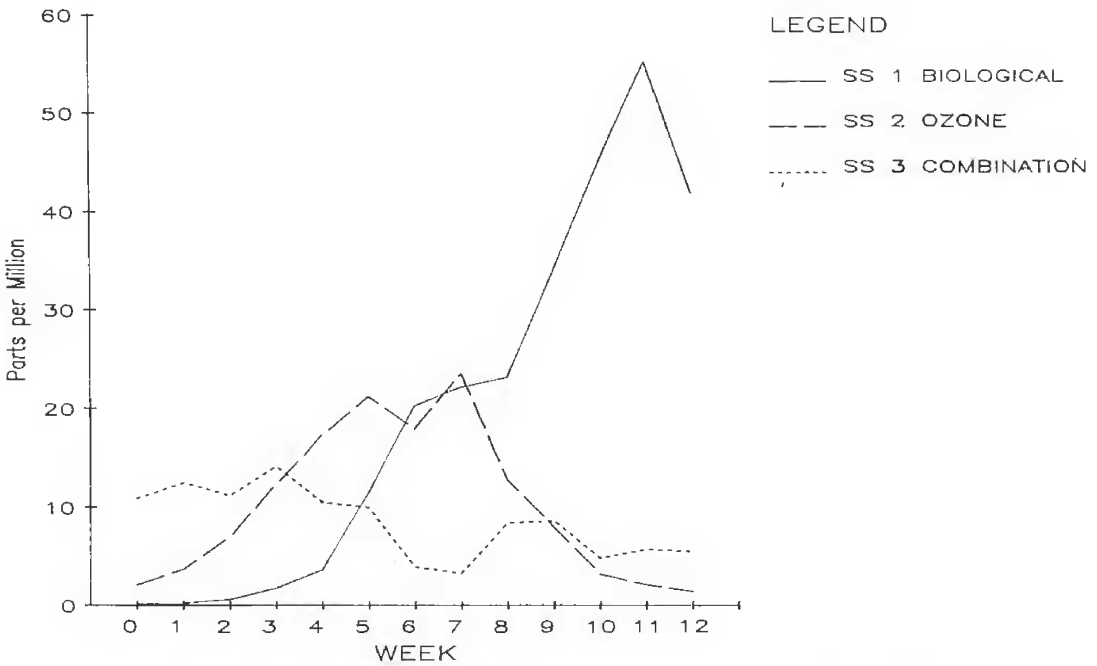


Figure 8. Graph of nitrite monitored over a 12-week period for three shrimp systems.

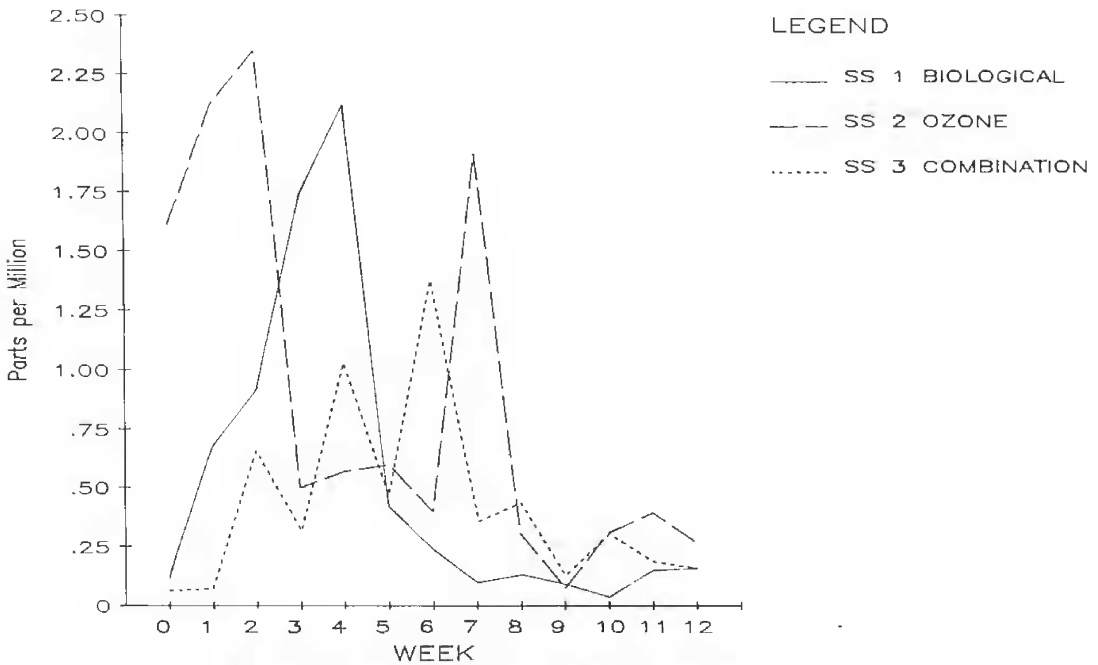


Figure 9. Graph of nitrate monitored over a 12-week period for three shrimp systems.



concentration, while the skimmer for SS-3a and 3b reduced the ammonia level. Ammonia was removed by the venturi in SS-2a and by the filter in SS-3a (Table 2). Nitrate was removed at a rate of 0.26 to 0.81 ppm from all systems except SS-2a. Most removal of nitrate from the systems occurred in the lamellar separator (Table 3).

The heterotrophic bacterial levels remained about the same throughout the study for both SS-1 and SS-2a (Fig. 10). Levels were basically the same for the two systems until week 11, when SS-1 was cleaned and a 80% water change was made. Although the bacterial level increased in SS-2a for this week, SS-1 levels decreased below the initial level. Numbers of presumptive *Vibrio* spp. decreased slightly for both systems through week 4 and increased through week 11. In all but one sample, (week 4), counts for the ozonated system were higher than for the biological system (Fig. 11). During two of the weeks, counts were considerably higher than initial levels.

#### DISCUSSION

Growth of *P. vannamei* in the closed systems was consistent with rates recorded for the species in various systems. Under experimental conditions, growth as high as

3 g/wk have been reported (Ogle 1992). At higher stocking densities, Reid (1989) reported growth of 0.57 and 0.61 g/wk (Table 4). Under commercial pond production with high flushing rates, *P. vannamei* growth rates of 0.27 to 1.85 g/wk have been recorded (Table 5). However, the target production period of 12 weeks resulted in 9 to 13 g animals. In order to achieve three crops per year, 1.8 g/wk growth will be necessary for reliable production of a 20 g animal in a 12-week growout period.

Survival rates were disappointing. Commercial ponds expect an 80-95% survival rate to make production feasible. In SS-1, SS-2a and SS-3a, numerous shrimp were lost when they jumped out of the culture tanks and escaped into other parts of the system.

Bacterial levels were expected to be minimal in SS-2a with ozone treatment. Actually, heterotrophic bacteria counts were higher in SS-2a than in SS-1 for most of the 12-week period. Apparently, ozone was being consumed in the system by the high solid organic content. Before repeating this study, modifications should be made to remove solids from the system.

Some biological filtration and denitrification was occurring in the lamellar. The nitrification occurring in the pump was unexpected and suggests that oxidation may have taken place due to air entrapment.

TABLE 2

Percent change in total ammonia between inflow and outflow of different filter components for four closed systems.

System	2a	2b	3a	3b
Pump	-13.5	-8.9	-15.5	-3.2
Lamellar	-8.5	-2.2	+3.2	-6.3
Cyclone				-1.5
Skimmer (O <sub>3</sub> )	+19.7	+4.1	-4.0	-7.0
Filter			-17.7	-12.2
Venturi (O <sub>3</sub> )	-25.2	-12.8		
Reservoir			-26.8	
Total ppm	-0.043	-0.050	-0.30	-0.047

All numbers based on an average of three samples.

TABLE 3

Percent change in nitrate levels between the inflow and outflow of different filter components for four closed systems.

System	2a	2b	3a	3b
Pump	-15.7	+0.2	+2.7	-0.6
Lamellar	+9.5	-2.8	-23.7	-19.8
Hydrocyclone				-1.0
Skimmer (O <sub>3</sub> )	+3.4	+1.7	+1.9	-3.9
Filter			-2.0	+1.0
Venturi (O <sub>3</sub> )	+19.4	0.0		
Reservoir			+15.4	
Total ppm	+1.28	-0.26	-0.37	0.81

All numbers based on average of three samples.

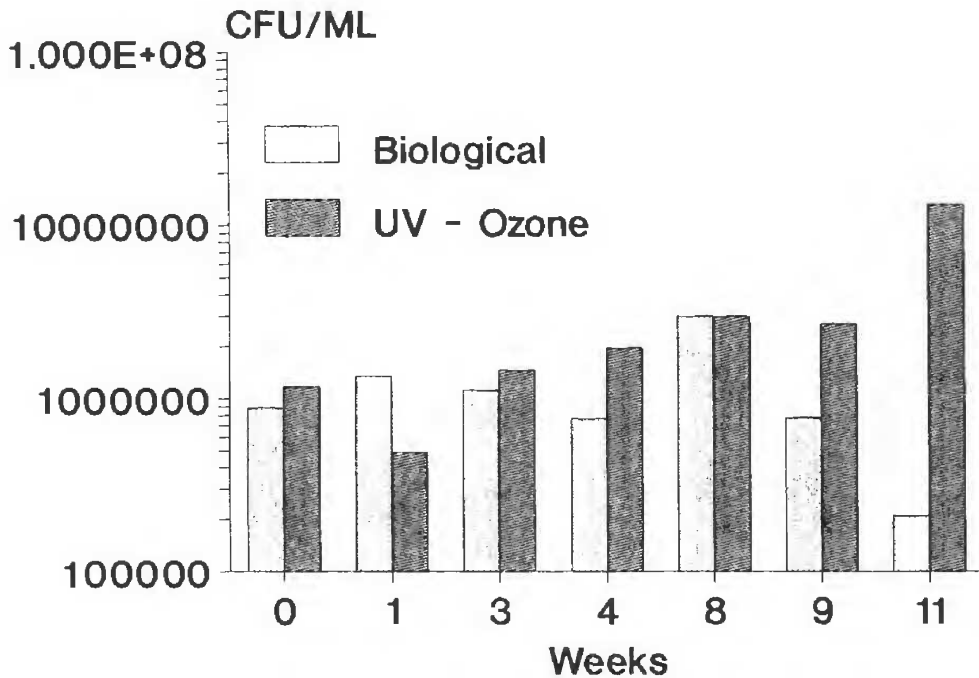


Figure 10. Total heterotrophic bacteria determined weekly for SS-1 and SS-2a.

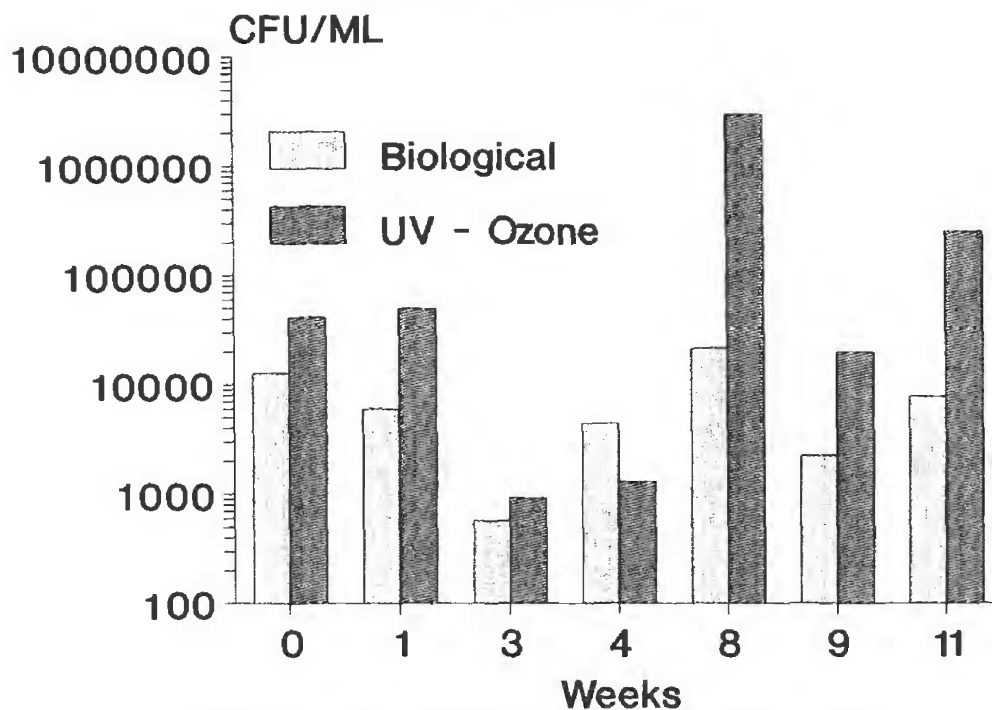


Figure 11. Presumptive *Vibrio* spp. sampled weekly for SS-1 and SS-2a.

TABLE 4  
Closed system growth (g/wk) of penaeid shrimp

Species	Minimum	Maximum	References
<i>P. aztecus</i>	0.34	0.55	Forster and Beard 1974
	0.20	0.53	Mock, Ross and Salser 1977
<i>P. indicus</i>	0.60	0.83	Forster and Beard 1974
<i>P. japonicus</i>	0.55	0.80	Forster and Beard 1974
<i>P. merguensis</i>	0.33	0.55	Beard, et al. 1977
<i>P. monodon</i>	0.80	1.58	Forster and Beard 1974
<i>P. occidentalis</i>	0.57	0.78	Forster and Beard 1974
<i>P. orientalis</i>	0.91	1.42	Forster and Beard 1974
<i>P. setiferus</i>	0.32	0.42	Forster and Beard 1974
	0.25	0.52	Mock, Neal and Salser 1973
<i>P. stylirostris</i>	0.12	0.39	Kennedy 1980
<i>P. vannamei</i>	0.57	0.61	Reid 1989
	0.59	0.99	Ogle 1992

TABLE 5  
Growth of *Penaeus vannamei* at various densities.

	System Size	System Type	Density #/m <sup>2</sup>	Growth g/wk
Wyban and Sweeney 1990	330 m <sup>2</sup>	pond	45	1.4
			75	1.75
			100	1.4
			150	1.07
Aquacop 1989	1000 m <sup>2</sup>	pond	139	0.60, 0.72
Reid 1989	—	closed	970	0.57
			1539	0.61
Ogle (unpublished data)	100 m <sup>2</sup>	pond	100	0.38
			16	0.80
			1	1.85
Ogle 1992	1.8 m <sup>2</sup>	tank	6.5	3.29
			13.7	2.31
			27.3	1.40
This report		closed	200	0.87, 0.99
			367	0.59
Sandifer, et al. 1987	28 m <sup>2</sup>	tank	10	1.41*
			20	1.35*
			40	1.11*
Sandifer, et al. 1988	0.25 ha	pond	12	0.94*
			42.5	0.84*
			20	0.56**
			40	0.52**
			60	0.51**
			100	0.57**
Trimble, W. 1980	0.08 ha	pond	2.5	1.8

\*calculated, \*\*estimated for 166-day growout

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